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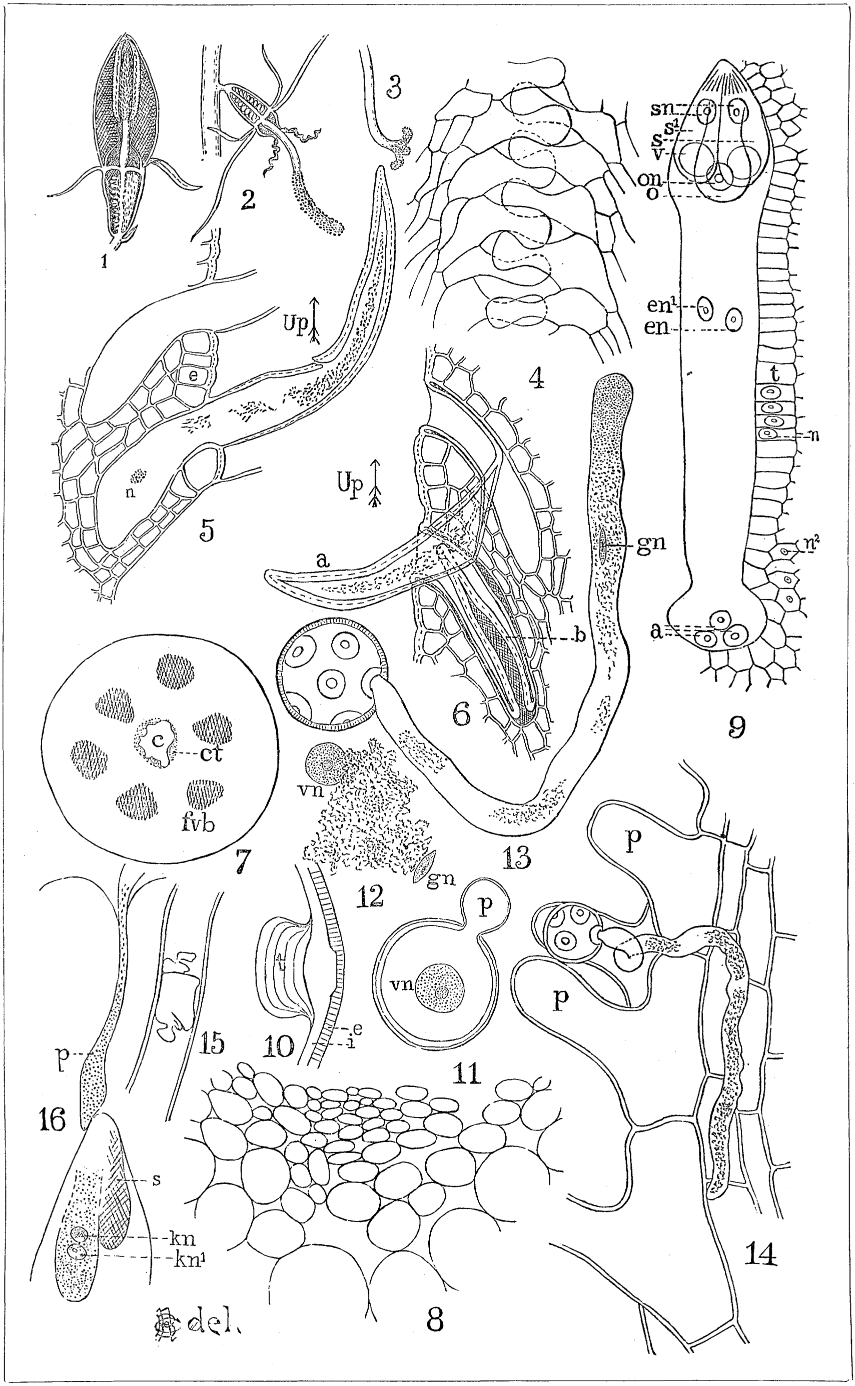

BARNES ON CAMPANULA.

The Process of Fertilization in Campanula Americana L.[1]

BY CHARLES R. BARNES.

(WITH PLATE X.)

I have undertaken to investigate some of the more recondite facts in the process of fertilization of *Campanula Americana* in order to increase the number of dicotyledons that have been so studied and to bring to light any peculiar adaptations which might exist.

The investigation of dicotyledonous plants is much more difficult than that of monocotyledons, because the transparence of the ovules and the usually large size of the nuclei which are so common among the latter seldom coexist in any dicot. *Campanula Americana* presents peculiar difficulties in the remarkable opacity of the ovules, though the nuclei are of moderate size.

As preliminary to an account of the study of fertilization, I proceed to give a brief account of the arrangement for cross-pollination which exist in this species.

The plant furnishes a marked example of proterandry, the anthers ripening and discharging their pollen in the unopened bud. The style at this time is not as long as the anthers, and that part in contact with the bursted thecæ is thickly clothed with stiff hairs pointing upward (*fig.* 1). The rapid growth of the style pushes these hairs, like the bristles of a bottle brush, through the thecæ and clears them of all the pollen, which adheres to the style of the now open flower (*fig.* 2). At this time the three lobes of the stigma are not manifest. They do not separate for some time afterward (*fig.* 3). The flowers are visited by wasps and bumblebees, the latter of which in crawling around the almost rotate corolla bring some part of the body, most commonly the hairy thorax or the legs, in contact with the pollen-laden styles of lately-opened flowers or the stigmatic surfaces of older ones. The nectar is secreted in abundance by a series of glands arranged in a circle on the flattened top of the ovary, to reach which the bee has to thrust his tongue between the style and the connivent, broadened bases of the filaments.[2] There is in these plants hardly a possibility of close-fertilization, the pollen being always brushed or blown off the style before the stigmas are at all exposed.

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[1] Read before the American Association for the Advancement of Science, August, 1885.

[2] Cf. Gray, Struct. Bot. p. 222, on proterandry of *C. rapunculoides*.

The essential points in regard to fertilization are as follows:

The development of the pollen is quite normal. There appear in each transverse section of the anther from six to eight mother-cells, which have somewhat thickened walls but do not float in liquid. The tapetal cells are unusually large and the three outer rows, particularly the inner, much smaller.

The stigmatic surfaces of the style are covered with nipple-shaped papillæ, the uppermost of which develop sooner than the lower. Those on any lobe interlock with those on the other two and thus prevent the premature separation of the lobes (*fig.* 4). As the growth of the outer surfaces of the style-lobes produces an increasing tension, the lobes finally separate. This separation begins at the base and the tips hold till the stigma is completely mature.

The hairs which brush out the pollen from the anthers (*fig.* 5) have their bases, *b*, deeply sunk in the tissues of the style. They are single-celled. Their tips, *t*, become quite thick and extensively cutinized, and the inner thickening layer is highly refractive. Just at the point of emergence the wall is thinner than at any other place. After these hairs, which are at first rigid, have served their purpose, they become softened and are in some way—I suppose by the contact of insects with the style—telescoped[3], so that the tip is left slightly projecting at the entrance of the pit thus formed (*fig.* 6). The result is as though one grasped the tip of a glove-finger and thrust it in upon itself until only the tip appeared projecting from the hand of the glove. This introversion of the hairs frees the pollen so that it is readily brushed off and carried away. Sometimes a pollen-spore drops into one of the pits and remains as a stopper for it. In the young hairs a beautiful streaming of the protoplasm is easily demonstrated[4].

The style has a small canal in the center, around which the conducting tissue is disposed in a variable number of strands of different sizes (*fig.* 7). Midway between the canal and the periphery are (usually) six fibro-vascular bundles. The conducting tissue, *ct*, is quite small in amount and sharply distinguished from the remaining tissue of the style (*fig.* 8).

The ovules are small, flattened and anatropous, with a *very short* funiculus, so that the micropyle is brought close against the placenta. The embryo-sac is straight and lies in the axis of the ovule (*fig.* 9). It is sharp-pointed at its micropylar end, somewhat larger in its upper fourth than elsewhere, and ends at the base in a considerable and abrupt enlargement. The tapetal cells, *t*, are unusually large and appear, in longitudinal section, columnar. In each lies a nucleus which is almost as wide as the cell. At both ends of the embryo-sac the tapetal cells become like the other cells of the nucellus. In the basal enlargement of the embryo-sac lie the three antipodal cells, *a*, one of which is usually a short distance above the other two. There are frequently two nuclei, *en*, en^1, belonging to the embryo-sac, near its middle. The pointed apex of the sac is occupied by the synergidæ, *s*, s^1, which are correspondingly long, nucleated near their upper ends and vacuolated near their lower. A little below the lower ends of the synergidæ lies the oosphere, *o*. It is noticeably more granular than the synergidæ and its nucleus is somewhat larger. A layer of protoplasm lines the embryo-sac and bridles extend in various directions between the egg-apparatus, the nuclei of the embryo-sac and the antipodal cells.

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[3] Müller, Fertilization of Flowers, p. 366–7, states that in the genus *Campanula* the hairs shrivel. This is not true for this species, nor *C. rapunculoides*, *fide* Strasburger, Bot. Practicum, p. 103.

[4] Cf. Strasburger's account of similar hairs in *C. rapunculoides*, l. c.

The mature pollen-spore is marked externally with numerous minute elevations (*fig.* 10), hardly more than thickenings of the cuticle, and 3–12 thin spots for the exit of the pollen tubes. At each thin spot in the extine there is a corresponding thickening of the intine.

The mature pollen-spore possesses two nuclei, the larger round one, the vegetative[5] nucleus (*vn*, *fig.* 11), occupying the center of the cell, while the spindle-form, generative nucleus lies to one side. I have been able to demonstrate both of these nuclei in only one case, when, after staining with borax-carmine, I crushed the cells (*fig.* 12). In several cases I have found the vegetative nucleus after treatment with acetic-methyl-green, and in other cases I have seen the generative nucleus in the pollen tubes (*fig.* 13). The difficulty of distinguishing the nuclei in this species is greatly enhanced by the markings on the walls, which simulate a nucleus and nucleolus. Strasburger states[6] that in *C. rotundifolia* the generative nucleus is easily demonstrated after staining with acetic-iodine-green, but it is not so in this species.

I have germinated the pollen-spores in ten per cent. sugar solution, and have obtained tubes of considerable length. In these cultivated tubes I have demonstrated both the vegetative and generative nuclei. The vegetative nucleus is therefore not destroyed in the spore, but passes early into the tube, where it soon undergoes disorganization. I have seen this nucleus in various stages of destruction. The generative nucleus passes later into tube; indeed it is usually among the last of the contents to leave the spore. I am not able to say whether or not it divides; presumably it does,[7] though I have seen but one such nucleus.

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[5] In this nomenclature I follow Strasburger (*Neue Untersuchungen*, p. 5), which is exactly the reverse of that generally used and even the reverse of that given by Strasburger in *Bot. Practicum*, p. 495.

[6] *Neue Unters.* p. 34.

When the pollen tubes are emitted on the stigma they sometimes pass straight and sometimes after turning upon themselves downwards *between*, and not *into*, the bases of the papillæ (*fig*. 14). The conducting tissue runs close beneath the stigmatic surfaces. In this tissue I have traced the tubes for several millimeters. The pollen tubes penetrate the strands of conducting tissue and do not enter the canal of the style. A short distance behind the apex of the tubes cellulose plugs are successively formed (*fig*. 15). These plugs, which have sometimes considerable length, are very prominent objects in longitudinal sections of the style or when the conducting tissue is teased with needles. The latter method permits one to trace the tubes for long distances. The pollen tubes pass down the style and follow the placentæ. When they emerge from a placenta they either enter the nearest micropyle at once or pass further, adhering *very closely* to the surface of the placenta.

I have detected the pollen tubes in a number of micropyles. The difficulty of tracing their further course is greatly enhanced by the opacity of the ovules and the consequent necessity of adopting the section method, as hereafter explained. I have been fortunate enough to find one specimen in which the pollen tube had entered the micropyle and penetrated to the synergidæ (*fig*. 16). In this specimen fertilization had taken place. One of the synergidæ at least (I could not see the other) had become disorganized and there were two nuclei in the oosphere. I am not certain that these were the male and female pronuclei, though they may have been. I am rather inclined to think from the considerable elongation of the oosphere that the embryonal nucleus had just divided preparatory to the formation of the first two cells of the suspensor. After fertilization the embryo-sac enlarges greatly, particularly in its middle third, and the formation of endosperm proceeds at once and rapidly.

The main points established regarding the fertilization of *Campanula Americana* are these:

The tapetal cells of the anther and ovule are unusually large.

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[7] Strasburger, l. c., p. 15, states that such division is the rule. I have not yet assured myself that it takes place in this species.

The pollen-spore possesses two nuclei, one of which, the smaller, persists and either with or without division copulates with the female pronucleus.

The pollen tube penetrates between the cells of the stigma and passes down the conducting tissue and not in the canal of the style.

There is the usual generative and vegetative apparatus in the embryo-sac.

METHODS.—For the study of the development of the pollen-spores I used alcohol-fixed buds, which had been twenty-four hours in alcohol-glycerine[8], commencing with those 2mm. in length. The sections of the entire bud were stained with methyl-blue.[9] The plant is an admirable one for the use of students in this respect.

For the study of the pollen-spores themselves fresh material is requisite. The best results were obtained by staining with borax-carmine.[10] The spores are placed in a drop of 2 per cent. acetic acid and after a few minutes a drop of borax-carmine added. This is allowed to remain an hour, the slide being protected from evaporation meanwhile. The stain is then washed out with acidulated alcohol[11] and a drop of dilute glycerine placed on the specimens.[12] The demonstration of the nuclei is extremely difficult.

The spores were germinated in a hanging drop of 3-12 per cent. sugar solution in the usual moist chamber. After three hours they were examined, the cover-glass with the drop being lifted off and allowed to fall on (1) a drop of acetic-iodine-green[13] or (2) a drop of picro-carmine.[14] After a few minutes dilute glycerine is run under the cover. Both yield excellent results. The nuclei in the tubes are thus more deeply stained than the cytoplasm.

Longitudinal sections of the stigmas serve for the study of the entrance of the pollen tubes. I used alcoholic material, without any staining, mounted in glycerine.

The pollen tubes in the conducting tissue may be studied either in longitudinal sections of the style or by laying open the style and drawing a needle through the canal, thus dragging out the conducting tissue. In the latter case care must be taken to tangle the strands as little as possible and methyl-blue should be used as a stain, otherwise the transparency of the pollen tubes renders them very difficult to follow. The very greatly elongated cells of the conducting tissue are almost exactly the diameter of the pollen tubes and are liable to mislead, were it not for the abundant cellulose plugs which occur only in the tubes.

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[8] Equal parts of 95 per cent. alcohol and glycerine.

[9] Aqueous solution.

[10] Grenacher's. See Bot. Pract., p. 630, or Archiv. f. mikr. Anat. xvi, 466.

[11] 70 per cent. alcohol. 100cc. HCl, 5cc.

[12] Strasburger. Neue Untersuchungen. p. 7.

[13] A drop of 1 per cent. acetic acid to which a small drop of iodine-green is added Strasburger, l. c., p. 6.

[14] Bot. Pract., p. 630.

In the study of the ovules material fixed in strong alcohol, in picric acid[15] and in chrom-acetic acid[16] was used. The contraction of the contents of the embryo-sac is unavoidable. I think the alcoholic material is quite equal to the others and less troublesome. I found it necessary to depend on getting chance sections of the ovules by cutting the whole ovary longitudinally and laying the sections in glycerine. Previous to the cutting the material is placed in alcohol-glycerine for twenty-four hours or more. After being mounted in glycerine the sections become clearer and clearer. I also tried cutting sections in various known directions by imbedding the ovules in colored pith to render them more easily seen. The results, on the whole, are not better than by depending on chance sections, and they are much more troublesome.

Explanation of Plate X.—Fig. 1, A bud bisected in the plane of the axis and bract. × 2. Fig. 2, diagram, an open flower bisected in the same plane; the stigmas unopened and the style laden with pollen. Fig. 3, a style with stigmas open. Fig. 4, interlocking papillæ of stigmas. × 195. Fig. 5, section of style from bud, showing hair; near *n* the remains of the nucleus. × 195. Fig. 6, section of style from which the pollen has been removed; *a*, a hair partly turned out of place; *b*, a hair in normal position. × 195. Fig. 7, transverse section of style; *c* lies in the canal; *ct* (shaded) the conducting tissue; *frb*, fibro-vascular bundle. × 37. Fig. 8, transverse section conducting tissue. × 356. Fig. 9, diagram of embryo-sac; *a*, antipodal cells; *en*, en^1, nuclei of embryo sac; n^1, nucleus of tapetal cell; n^2, nucleus of a parenchyma cell of the nucellus; *o*, oosphere; *on*, nucleus of oosphere; *s*, s^1, synergidæ; *sn*, nucleus of synergida *s*; *t*, tapetal cells; *v*, vacuole of synergida s^1. Fig. 10, diagram, optical section of wall of pollen-spore through one of the exit spots; *e*, extine; *i*, intine; *t*, stratified local thickening of intine. Fig. 11, a pollen spore, showing vegetative nucleus, *vn*; *p*, a beginning pollen tube. × 356. Fig. 12, part of the contents of a crushed pollen-spore; *gn*, generative nucleus; *vn*, vegetative nucleus. × 356. Fig. 13, a cultivated pollen tube; *gn*, generative nucleus. × 356. Fig. 14, a pollen tube penetrating the stigma; *p*, *p*, papillæ of stigma. × 260. Fig. 15, a cellulose plug in a pollen tube. × 380. Fig. 16, a pollen tube penetrating to the embryo-sac; *p*, pollen tube; *s*, synergida disorganized; *kn*, kn^1 (male and female pronuclei ?), the germ nucleus after its first division. × 356.

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[15] Saturated aqueous solution.

[16] Chromic acid, 0.7; acetic acid, 0.3; distilled water, 99. Strasburger, l. c., p. 328.